

WE CLAIM:

1. A method for immobilizing a biological molecule in a porous inorganic matrix,
5 said method comprising:
forming an aqueous composition comprising a ceramic oxide colloidal sol mixed
with an acidified oxide salt solution;
adding to said composition an amount of the biological molecule in a
physiologically acceptable-buffered solution, said aqueous composition becoming turbid
10 on being transformed into a polymerizing hydroxide solution and transforming to a gel;
shaping the gel produced in step (b) into a final form; and
aging the gel;
wherein said biological molecule is entrapped within pores of the gel, and the
activity of the biological molecule is retained.
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2. The method of claim 1, wherein the gel is aged for about two weeks.
3. The method of claim 1, wherein the gel is aged at a temperature of from about
40° C to about 40° C.
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4. The method of claim 1, wherein the pH of the mixture after the addition of the
biological molecule is between about 6.0 and about 8.5.
5. The method of claim 1, wherein the pH of the mixture after the addition of the
25 biological molecule is between about 4.0 and about 7.0.
6. The method of claim 1, wherein the pH of the mixture after the addition of the
biological molecule is between about 7.0 and 9.0.
- 30 7. The method of claim 1, further comprising crushing the aged gel into particulates.

8. The method of claim 7, wherein the crushed gel particulates are between about 10 μm and about 80 μm in diameter.

5 9. A method for immobilizing a biological molecule in a porous inorganic matrix, said method comprising:

forming an aqueous composition comprising a ceramic oxide colloidal sol and a dissolved metal silicate mixed with an acidified oxide salt solution;

10 adding to said composition an amount of the biological material in a physiologically acceptable-buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel; shaping the gel produced in step (b) into a final form; and aging the gel;

15 wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained.

10. The method of claim 9, wherein the sol is comprised of colloidal silica sol and a dissolved metal silicate.

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11. The method of claim 9, wherein the metal silicate is sodium silicate.

12. The method of claim 9, wherein the sol comprises a tetraalkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo.

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13. The method of claim 12, wherein the silane is substituted with a C₈-C₂₄ alkyl group.

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14. The method of claim 13, wherein the alkyl group is C₁₈.

15. The method of claim 12, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate (TEOS), tetra-methyl orthosilicate (TMOS), and combinations thereof.

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16. The method of claim 1, wherein the sol is comprised of colloidal silica sol and a dissolved metal silicate.

17. The method of claim 16, wherein the metal silicate is sodium silicate.

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18. The method of claim 1, wherein the sol comprises a tetraalkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo.

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19. The method of claim 18, wherein the silane is substituted with a C₈-C₂₄ alkyl group.

20. The method of claim 19, wherein the alkyl group is C₁₈.

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21. The method of claim 18, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate (TEOS), tetra-methyl orthosilicate (TMOS), and combinations thereof.

22. The method of claim 12, further comprising crushing the gel into particulates.

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23. The method of claim 22, wherein the crushed gel particulates are between about 10 μ m to about 80 μ m in diameter.

24. The method of claim 1, wherein the particle size of the ceramic oxide colloidal sol is selected to produce pores when the gel is aged, said pores being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

5 25. The method of claim 1, wherein the gel produced in step (b) is shaped into forms selected from the group consisting of a monolithic gel, thin film, or fiber.

26. The method of claim 24, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.

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27. The method of claim 1, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.

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28. The method of claim 9, wherein the particle size of the ceramic oxide colloidal sol is selected to produce pores when the gel is aged, said pores being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

29. The method of claim 28, wherein the diameter of the pores is less than the diameter of the entrapped biomolecule.

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30. The method of claim 9, wherein the gel produced in step (b) is shaped into forms selected from the group consisting of a monolithic gel, thin film, or fiber.

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31. The method of claim 9, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.

32. The method of claim 31, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.

33. The method of claim 24, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.

5 34. The method of claim 24, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

35. The method of claim 24, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

10 36. The method of claim 24, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

37. The method of claim 28, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.

15 38. The method of claim 28, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

20 39. The method of claim 28, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

40. The method of claim 28, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

25 41. The method of claim 1, wherein the biological molecule is selected from the group consisting of polynucleotides, enzymes, antibodies, coagulation modulators, cytokines, endorphins, peptidyl hormones, kinins, receptors, genes, gene fragments, cell fragments, membrane fragments, and solubilized membrane proteins.

42. The method of claim 41, wherein the enzyme is selected from the group consisting of RNase, DNase, telomerase, ligase, nuclease, ribonuclease; hydrogenase, dehydrogenase, aldase, amidase, aminotransferase, amylase, anhydrase, apyrase, arginase, aspartase, aspariginase, carboxylase, carboxypeptidase, catalase, cellulase, cholinesterase, acetylcholinesterase, deaminase, dextranase, dismutase, elastase, esterase, fumarase, glucosidase, hexokinase, isomerase, invertase, kinase, lactasee, lipase, lysozyme, malase, naringinase, oxidase, oxygenase, papain, pectinase, peptidase, pepsin, peroxidase, phosphodiesterase, phosphotase, protease, reductase, transferase, tyrosinase, urase, trypsin, chymotrypsin, hydrolases, isomerases, proteases, ligases and oxidoreductases such as esterases, phosphatases, glycosidases and peptidases, superoxide dismutase (SOD), tissue plasminogen activator (TPA), renin, adenosine deaminase, alpha-glucocerebrosidase, asparaginase, dornase-alpha, hyaluronidase, elastase, trypsin, thymidine kinase (TK), tryptophan hydroxylase, urokinase, kallikrein, bromelain, cathepsins B, D, G, C, clostripain, endoproteinase Arg C, endoproteinase Asp N, endoproteinase Glu C, endoproteinase Lys C, Factor Xa, proteinase K, subtilisin, thermolysin, acyloamino acid releasing enzyme, aminopeptidases, carboxypeptidases, and pyroglutamate aminopeptidase.

43. The method of claim 1, wherein the colloidal sol particle size is from about 1 nm to about 30 nm.

44. A method of preparing a microanalytical device, comprising forming a sol-gel comprising an entrapped biological molecule, crushing the sol-gel to particulates having a diameter of from about 10 μm to about 80 μm , and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device.

45. A method of preparing a microanalytical device comprising forming a sol-gel comprising an entrapped biological molecule, wherein the form of said sol-gel is selected from the group consisting of a monolithic gel, thin film, or fiber and wherein the sol-gel is placed in or on the microanalytical device.

46. A method of using a microanalytical device comprising a sol-gel comprising an entrapped biological molecule, comprising applying an analyte sample to the bed, optionally applying additional buffer solution to the bed, and analyzing the eluant from the bed.
47. The method of claim 44 or 45, wherein the bed on the microanalytical device is in the form of a microcolumn or microchannel.
48. The method of claim 44 or 45, wherein the bed on the microanalytical device is in the form of a microarray.
49. The method of claim 46, wherein the eluant is analyzed using mass spectrometry.
50. The method of claim 46, wherein the eluant is analyzed using micro or capillary electrophoresis.
51. The method of claim 46, wherein the interaction of any component in the sample with the entrapped biological molecule in the sol-gel is measured using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index (RI) and Raman spectroscopies.
52. The method of claim 46, further comprising washing the sol-gel with a solution to elute analytes from the sol-gel, and analyzing the analytes.
53. The method of claim 52, wherein the analytes are analyzed using mass spectrometry.

54. The method of claim 52, wherein the analytes are analyzed using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index (RI) and Raman spectroscopies.

5 55. The method of claims 44 or 45, wherein the microanalytical device is fabricated by a method selected from the group consisting of silicon micromachining, microlithography, molding and etching.

10 56. The method of claim 45, wherein the sol-gel is formed in situ on the microanalytical device.

15 57. In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature particulates of sol-gel having a diameter of from about 10 μm to about 80 μm .

20 58. In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel is in a form selected from the group consisting of a monolithic gel, a thin film, and a fiber.

25 59. The microanalytical device of claims 57 or 58, adapted for performing high throughput screening of samples.